REMARKS

Claims 1, 39, 41-46, 48-56, and 58-63 are pending. Claims 2-38, 40, 47 and 57 have been canceled. Claim 1 has been amended to clarify the subject matter encompassed by the claim and to incorporate the language of claim 47. Support for these amendments can be found at page 1, lines 18-23 and page 8, lines 8-11 and in original claim 47. Claim 39 has been amended to clarify the subject matter encompassed by the claim. Support for these amendments can be found at page 7, lines 13-16 and page 8, lines 8-11. Claim 50 has been amended to clarify the subject matter encompassed by the claim. Support for these amendments can be found in original claim 50. Claim 51 has been amended to clarify the subject matter encompassed by the claim. Support for these amendments can be found in original claim 51. Claim 52 has been amended to clarify the subject matter encompassed by the claim. Support for these amendments can be found in original claim 52. Claim 53 has been amended to clarify the subject matter encompassed by the claim. Support for these amendments can be found in original claim 53. Claim 54 has been amended to encompass the subject matter of original claim 47. Claim 58 has been amended to clarify the subject matter encompassed by the claim. Support for these amendments can be found at page 8, line31 to page 9, line 9. Claim 61 is new. Support for this claim can be found at page 8, lines 13-16. Claim 62 is new. Support for this claim can be found at page 8, lines 13-16. Claim 63 is new. Support for this claim can be found in original claim 1, table 3 and page 26, line 29- page 27, line 2. No new matter is added by entry of these amendments.

Claim Objections

The Examiner has objected to claim 1 because acronyms such as A/EEC, EPEC, etc. should be spelled out in the first instance in a chain of claims.

Applicants have amended claim 1 to spell out the objected to acronyms. Thus, Applicants request that the Examiner withdraw this rejection.

Rejections under 35 USC §112

The Examiner has rejected claims 1 and 39-54 under 35 USC § 112, second paragraph for allegedly being indefinite. More specifically, the Examiner alleges that:

- *it is unclear which genes the phrase "at least one primer..." is intending to encompass;*
- *the preamble of the claims is not commensurate with the steps of the method;*
- *the phrases "such as" and "such as at least" render the claims indefinite;*
- *a broad range together in the same claim with a narrow range is considered indefinite;*
- *the phrases "preferable" or "preferably" render the claims indefinite.*

Applicants have amended the claims to clarify the subject matter encompassed by the invention. Applicants believe that these amendments provide clarity without affecting the scope of the claims. Reconsideration and withdrawal of these rejections is requested.

Rejections under 35 USC §103

The Examiner has rejected claims 1 and 39-43, 45-49, 54-57 and 59-60 under 35 USC § 103(a) as allegedly being unpatentable over Toma et al (J. Clin Microbiol., 2003, Vol. 41(6):2669-2671) in view of Grabowski (WO 02/053771, corresponding to US Patent Pub. 2004/0110251).

More specifically, the Examiner alleges that Toma et al teach multiplex PCR assays for the identification of DEC species, by detecting the genes: eae, stx, elt, est, and ipaH. The Examiner admits that Toma et al do not teach amplification of exhA, vtx1 or vtx2. The Examiner further alleges that Grabowski et al teach multiplex PCR assays for detecting the genes: vtx1, vtx2, eae and hlyA (exhA). The Examiner asserts that one of skill in the art would have been motivated to combine the methods taught by Toma and Grabowski "when both teach performing multiplex PCR with two or more primers in a single reaction for the identification of specific genes."

Applicants respectfully traverse this rejection. The combination of Toma and Grabowski does not teach or render obvious Applicants' invention.

Contrary to the Examiner's assertion, it would not be obvious to one of skill in the art to combine the methods of Toma with those of Grabowski. While Toma describes amplification of some of the genes amplified in Applicants' invention, and Grabowski describes others, it is the simultaneous amplification and detection of the specific combination of genes claimed by Applicants that renders the screening method effective. It is not proper for the Examiner to pick and choose some genes from each of the various references to arrive at Applicants' method, as it uses impermissible hindsight using knowledge gleaned from Applicants' invention.

In addition, it is not proper to combine Toma and Grabowski as one cannot simply combine the primers used by Toma with the primers used by Grabowski to get to Applicants' invention. The particular combination of primers used in the method is not a random combination of *any* primers which amplify each of the selected genes. Rather, the particular selection of primers is critical as they must be capable of functioning under identical PCR conditions producing gene fragments that are separable in a robust assay. Therefore, it is not straightforward for one of skill in the art to merely combine the primers of Toma with the primers of Grabowski to get a functioning multiplex PCR assay. Rather, the primers must be designed specifically for the intended assay. Here, that refers the simultaneous detection of all of the genes *ipaH, eae, sta/estA, vtx1, vtx2,* and *elt.*

Further, sensitivity and specificity of the method are important aspects in detection of pathogenic microorganisms, since the template concentration is often very low in samples (e.g., in feces samples), and the number of non-target microorganisms is very high. Applicants' primers disclosed in Table 3 have proven superior without losing specificity. The specificity is very important as DEC have multiple variants of each gene and it is therefore important that the primers/probes detect a conserved region of the gene in question. As shown in Table 5 of the specification, testing with all of the WHO reference strains has shown that the primers and probes of the instant invention are

specific. Neither Toma nor Grabowski describes any of the specific primers disclosed in Applicants' Table 3.

Thus, the claimed invention provides advantages not contemplated by the cited prior art and a method of achieving a result not possible based on the teachings of the art. Reconsideration and withdrawal of the rejection is requested.

The Examiner has rejected claims 44, 50-53 and 58 under 35 USC § 103(a) as allegedly being unpatentable over Toma et al (J. Clin Microbiol., 2003, Vol. 41(6):2669-2671) in view of Grabowski (WO/053771, corresponding to US Patent Pub. 2004/0110251) and in further view of Karube et al (WO98/50581, corresponding to US 6,391,546).

The Examiner admits that Toma and Grabowski do not teach the use of probe sequences from Table 7. However, the Examiner alleges that Karube describes a method of detecting target nucleotide sequences, and that Karube teach a sequence having 100% sequence identity to the instant SEQ ID NO: 32. Thus, the Examiner asserts, it would be prima facie obvious for one of skill in the art to combine the methods of Toma and Grabowski and incorporate additional probes as taught by Karube.

Applicants respectfully traverse this rejection. Karube adds nothing to the combination of Toma and Grabowski to render the instant invention obvious.

The Examiner asserts that Karube teaches a sequence having 100% identity with SEQ ID NO: 32. However, this sequence is a part of a much larger "DNA sequence coding for Type II verotoxin of pathogenic Escherichia coli O-157"[1]. It is clear to one of skill in the art that if one were probing for type II verotoxin in a method such as the one described in Applicants' invention, one would have to use a portion of the DNA sequence encoding type II verotoxin to do so. However, the germane issue is which sequence should be used.

Applicants' do not claim that the sequence of type II verotoxin is novel or encompassed by the instant invention. Rather, Applicants have specifically designed

[1] Karube, column 1, lines 66-67, and Fig 4 (using US 6,391,546 as the reference)

primers and probes which amplify and detect a specific set of genes under the same set of conditions, e.g., in a multiplex PCR reaction. Disclosure of the entire type II verotoxin sequence does not render the instant invention obvious, because, as is more fully described above, it is the selection of the specific region of the gene to be used as a template for the primer/probe sequence that is critical. For example, selection of the wrong portion of the DNA sequence to use as a probe will not be effective as it will not hybridize to all variants of the gene. While the Karube document contains the DNA sequence of type II verotoxin, the specific region described by Applicants as SEQ ID NO: 32 is not identified as the probe sequence used to detect *vtx2* by Karube[2]. Thus, Karube adds nothing to Toma and Grabowski to teach or suggest the specific combination of genes, primers and probes claimed in the instant invention.

Reconsideration and withdrawal of the rejection is requested.

Information Disclosure Statement

Applicants submit concurrently with the filing of this paper, an Information Disclosure Statement which re-submits document Q of the IDS filed April 12, 2006 in its entirety. Applicants request that the Examiner consider this document during the course of prosecution.

[2] *Id.* at Fig 4.

The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees due to our Deposit Account, Number 08-3040.

Respectfully submitted,

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